Stressor marrow response in the case of neonatal sepsis-significance of nRBCs in peripheral smears

Running title: nRBCs and neonatal sepsis

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Abstract

Background: Nucleated red blood cells (nRBC) are immature erythrocytes whose production is thought to be driven primarily by the interplay of hypoxia and erythropoietin (EPO) synthesis. It is classified as early onset neonatal sepsis which occurs with first 24 hours of life, or late onset neonatal sepsis which occurs after 48-72 hour. The aim of this study is analysing the cause for the presence of nRBCs in peripheral blood of neonates sent for routine workup in the Hematology Laboratory of the Department of Pathology, SMSR, Sharda University.

Method: Retrospective analysis of 27 complete blood count (CBC) and peripheral blood smears (PBS) of neonates was done of data of one month where peripheral smears had been made and stored for examination and where reports for peripheral smear had been dispatched. Clinical details (as available from clinician), parameters for neonatal sepsis (NLR, PLR, IG), platelet counts and nRBC counts recorded and tabulated. For result tabulation we divided the neonatal samples into two groups 0-1 day and 2- 30 days.

Results: The neonates were 17 males and 9 females. Assessment of the NLR, PLR and IG were indicated that it was more in the group 2-30 days as compared to cases 0-1 day old. Moreover, nRBCs were also raised in group 2-13 days old.

Conclusion: We conclude that finding nRBCs in peripheral blood smear is an important indicator of neonatal stress, including sepsis, jaundice and hypoxic sepsis.

Keywords: nRBC, Neonatal sepsis, Stressor, Response

Introduction

Neonatal sepsis is a life-threatening condition caused by systemic bacterial, viral or fungal infection with the first 28 days of life. It is associated with hemodynamic changes and other clinical manifestations and result in neonatal morbidity and mortality. Nucleated red blood cells (nRBC) are immature erythrocytes whose production is thought to be driven primarily by the interplay of hypoxia and erythropoietin (EPO) synthesis (1-2). The clinical presentation of neonatal sepsis is non-specific. This includes symptoms like fever, refusal to feed, respiratory distress, lethargy, irritability, convulsions, bulging fontanels, abdominal distensions and temperature dysregulations. It is classified as early onset neonatal sepsis (occurring with first 24 hours of life) or late onset neonatal sepsis (occurring after 48-72 hour) (3, 4).

There are multiple markers for neonatal sepsis in hematology and biochemistry. There is a defined hemogram criterion for diagnosis of sepsis. Some of the indicators established as well as under study are Total Leucocyte Count (TLC), Absolute Neutrophil Count (ANC), Immature Neutrophil Count, Immature to Total Neutrophil Count (I/T) ratio, Platelet Count (PLT), Mean Platelet Value (MPV) and Platelet Distribution Width (PDW). A Complete Blood Count (CBC) and differential leucocyte count may be done to determine changes associated with the infection (eg, thrombocytopenia or neutropenia) or to monitor the development of a left shift or changes in the ratio of immature to total neutrophils, although the sensitivity and specificity of these markers is low (5, 6). Serial monitoring of the CBC may be useful in aiding the differentiation of sepsis from nonspecific abnormalities due to the stress of delivery (2,3).

The presence of nRBCs is a significant finding in peripheral smears of cases of neonatal sepsis, indicating a stress response of the marrow. We present a series of cases of neonates, with data presented as original research work, highlighting the presence of nRBCs in cases of neonatal sepsis.

Method

Retrospective analysis of 27 CBC and peripheral blood smears (PBS) of neonates was done of data of one month, where peripheral smears had been made and stored for examination, and where reports for peripheral smear had been dispatched. Clinical details (as available from clinician), parameters for neonatal sepsis (NLR, PLR, IG), platelet counts and nRBC counts recorded and tabulated. For result tabulation, we divided the neonatal samples into two groups 0-1 day and 2- 30 days.

Results

Retrospective data with clinical history were collected from records available in the Department of Pathology archives as well as archives available in the medical records department for clinical details. Data was collected for all cases having nRBCs in CBC and peripheral smears exceeding a count of 10nRBC/100 WBCs. NLR and PLR were calculated from CBC, and IG were calculated from data available in CBC and peripheral smears.

The study analyzed 26 cases, of which 17 were males and 9 were females; all neonates were between 0 and 30 days of age. Demographic details of the cases taken in the study. Table 1 highlights the age-related findings (as per days) of CBC parameters under study in neonates 0-1 day and 2-30 days. An average of individual parameters was calculated, and it was noted that between 0-1 day, an average of 11.6 nRBCs was observed in smears, while 39.6 nRBCs were observed in neonates aged 2-30 days.

Table 1. Age correlation with CBC-derived parameters seen in neonatal cases

Age (Day)	**NLR**	**PLR**	**IG**	**nRBC**
0-1	1.26	0.05	3.06	11.6
2-30	3.81	0.05	5.36	39.6

The table indicated the average values of CBC derived parameters as well as percentage of immature granulocytes divided as per patient age. This was a descriptive analysis highlighting the average values of parameters in study.

Table 2. Correlation of pathological parameters with Clinical parameters

Parameters	Hypoxia	Sepsis	Jaundice
nRBC	**30.45**	11.24	8.4
NLR	1.1	1.9	3.3
PLR	0.045	0.043	**0.072**
IG	1.9	5.3	3.42

Table 2 shows correlation of pathological parameters where the numbers highlight an average percentage of cases for example 30.45% of cases with hypoxia shows nRBCs,11.24 % with sepsis shows nRBCs, 8.4% with neonatal jaundice shows nRBCs and 50.08% cases had a combination of clinical presentations like jaundice with sepsis, hypoxic sepsis etc and showed nRBCs in the smears.
A significant NLR ratio (6.3) was seen in cases where sepsis, hypoxia or jaundice co-existed as compared to single entities like hypoxia, sepsis, jaundice.
PLR ratio was significant in cases with jaundice with nRBC in smear as compared to other cases. Immature granulocytes (IG) were seen more (10.62) in cases with mixed clinical features.

Discussion

Sepsis is generally viewed as a disease aggravated by an inappropriate immune response (7,8). Previous studies showed that the inflammatory response, characterized by cytokine release, is accompanied by an increased nRBC production (9). Studies have found that fetuses delivered with fetal distress have normal EPO levels and elevated IL-6 concentrations (10). This implies that the fetal inflammatory response and fetal stress may have distinct roles in NRBC production and/or release in peripheral circulation. In our study we saw that a combination of hypoxic sepsis as well as infection was potent stimulator for release of nRBCs in the peripheral smears.
nRBCs are normally present in fetal circulation but then disappear in the first postnatal month in healthy neonates (11). nRBC counts vary by gestational and chronologic age (12). In infants, nRBCs emerge from the bone marrow approximately 28 h after a stressor such as hypoxia (13). Elevated nRBCs have been characterized as a signal of intrauterine and early postnatal stress among newborns, and they are associated with events such as fetal acidemia, meconium passage during delivery, and perinatal complications (14). In our study we saw raised IG levels in cases where nRBCs were present in large numbers supporting the finding that infections lead to stressor response in neonates.
nRBCs have been evaluated as a prognostic marker for outcomes following perinatal hypoxia. For infants who experience hypoxic ischemic encephalopathy, elevated nRBCs were associated with increased risk of immediate post-natal complications, and elevated nRBC counts within the first 6 h of life. In one study combination of electroencephalogram (EEG) and nRBC counts have demonstrated improvement over EEG alone for determining prognosis and outcomes of hypoxic ischemic encephalopathy (15).
In our case the NLR was more in cases of mixed clinical picture while PLR was more in deranged liver functions presenting as neonatal jaundice. nRBCs were raised mostly in mixed picture of clinical symptoms followed by hypoxic sepsis. Compiling clinical and pathological findings together it can be said that instead of sepsis induced by infection the neonate's life-threatening condition could be attributed to birth asphyxia/hypoxia, however no documented record was available for the same.
Our finding supports the current line of thought that increased nRBC production in the immediate neonatal state primarily reflects hypoxic injury (16). Much of the existing literature focuses on hypoxia's contribution to elevated EPO levels, and thus, NRBC counts. Several studies have shown that in cases of classic chronic intrauterine fetal stress, such as preeclampsia and intrauterine growth restriction (IUGR), fetal serum and amniotic fluid EPO levels are elevated (7-12). Such a relationship has led to the prevailing idea that the NRBC count *in utero*, as in the adult state, is mainly driven by hypoxia-triggered EPO release.
nRBCs are in the peripheral blood of normal infants up to the fifth day of life. They are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as precursors to reticulocytes and mature erythrocytes. In one study, the sensitivity of nRBC for detecting sepsis was 35%, its specificity 53.48%, its positive predictive value and its negative predictive value were 23.07% and 67.64% respectively (17). Their findings were similar with the study done by Tripathi

et al (2010) (18, 19). They stated that activated macrophages releases cytokines which play important role in stimulating nRBC in absence of hypoxia. She also revealed that nRBC were significantly increased in early and late neonatal sepsis. Another study which was done by Dulay et al (2008) also stated significant increase in nRBC with early onset of neonatal sepsis (13).
In our case we saw up to intermediate normoblasts which are typically at orthochromic normoblastic stage of maturation, characterized by a round nucleus with markedly dense chromatin. The cytoplasm of the circulating nucleated red cells typically display polychromasia, and the cells are somewhat larger than mature erythrocytes, reflecting their more maturation stage. The marked increase in nRBC count gives rise to spuriously high WBC count. The appearance of nRBC signifies bone marrow damage or stress that potentially serious underlying disease and extramedullary hematopoiesis have been activated. In this case we did not go about calculating the standard CBC indicators of neonatal sepsis since our primary findings were of nRBCs and late and intermediate normoblasts in the smear examined.

Conclusion

We conclude that such an extreme manifestation of neonatal sepsis, with the main response by erythroid lineage spill over in peripheral blood, is rare and can be used as a soft indicator for the etiology of the disease to be interpreted.

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Ethical statement

The research study has been approved by the institutional ethics committee and is compliant with all necessary regulations. Approval for waiver from the institutional ethical committee has been taken (Ethical Code: SMSR/IEC/274 and applied for at PGICH,noida.)

Conflicts of interest

All contributing authors declare no conflicts of interest.

Author contributions

NT: Data collection, conceptualization, manuscript writing. RS: Data collection, manuscript editing. PS: Data collection, manuscript editing.

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Abbreviations

NLR: Neutrophil Lymphocyte Ratio
LMR: Lymphocyte Monocyte Ratio
IG: Immature Granulocyte
nRBC: nucleated Red Blood Cells
TLC: Total Leucocyte Count
PLR: Platelet Lymphocyte Ratio